Original article

Interleukin-12 levels in Egyptian children with type 1 diabetes mellitus

Background: *Type 1 diabetes mellitus (T1DM), arising through a complex interaction of immune, genetic and environmental factors, results from autoimmune destruction of insulin-producing β cells. Cytokines are critical to the function of both innate and adaptive immune responses. Interleukin-12 p40 production influences T cell response, and may therefore be important in T1DM pathogenesis.* ***Objective:*** *to study the changes in IL12 levels in children with T1DM.* ***Study design:*** *fifty T1DM children among those attending diabetes clinic at Zagazig University hospitals, were included in the study. They were 27 males and 23 females (mean age, 9.19 ± 3.3 years). Thirty age and sex matched healthy children were serving as a control group. All children were subjected to full history taking, physical examination, complete blood count (CBC), random blood sugar, glycated hemoglobin (HBA1C) and serum IL-12 levels assessed by ELISA.* ***Results:*** *Diabetic children had significantly higher white blood cell count, HBA1C, and IL12 levels than healthy children. While there was no effect of gender on IL12 levels, there were significant increase in IL12 levels in newly diagnosed cases, those with higher body mass index and those who had the poorest glycemic control.* ***Conclusion:*** *type 1 diabetes is associated with elevation of IL-12 levels. This association is more evident in both newly diagnosed and poorly controlled patients indicating a relevant role of IL-12 in the pathogenesis of the disease.*

Keywords: Type 1 Diabetes Mellitus, Interleukin 12, Egypt.

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INTRODUCTION

Diabetes mellitus type1 (T1DM) is characterized by the appearance of insulitis and the presence of β-cell autoantibodies[1]. Emerging evidence indicates that immune cell infiltrates comprised predominantly of CD8 and CD4 T cells, B cells, and macrophages are present in islets from newly diagnosed patients, implicating both innate and adaptive immune systems in the pro-inflammatory process leading to islet cell death[2] .

Cytokines have been reported to be involved in the immunopathology of several autoimmune diseases including type 1 diabetes .There is evidence that cytokines could have a direct role in β-cell death[3] .

Interleukin-12 p40 production influences T cell response, and may therefore be important in T1DM pathogenesis. Interleukin-12 (IL-12) drives the differentiation of T lymphocytes towards the Th1 subset, characterized by production of cytokines leading to cell mediated immunity. In addition, IL-12 is important in immune response to infections; however, it has been shown that in absence of infection, IL-12 induced autoreactive T cell responses might predispose to self-destructive immunity[4].

The significance of IL-12 in human autoimmunity is not clear, and serum levels of IL-12 in diabetes mellitus have not been well established. Elevated levels of this cytokine have been observed in most autoimmune diseases.[5,6]

METHODS

The study population consisted of 50 patients with type 1 diabetes mellitus (mean age, 9.19±3.3 years) recruited from the outpatient endocrinology clinic, Zagazig University Hospitals, during the period from August 2011 to September 2012. In addition, 30 age and sex matched healthy children (mean age, 8.7±3.8 years) with no family history of diabetes served as a control group.

Exclusion criteria: the study excluded patients with signs of chronic inflammatory, neoplastic, allergic or other autoimmune diseases, in addition to patients receiving immunomodulatory drugs.

Methodology: Diabetic children were residing in the vicinity of the Zagazig University Hospital and were treated with a standard dose of human insulin obtained from Novo Nordisk Industry, Copenhagen, Denmark. All patients were taking 2 to 4 subcutaneous insulin doses per day.
All children were subjected to: Full history taking to ensure the diagnosis of type 1 diabetes and reveal family history of the disease, thorough physical examination, routine laboratory investigations**:** complete blood count (CBC), random blood sugar, HBA_1C (glycated hemoglobin) and serum IL-12 levels were assessed by ELISA.

***Measuring HbA_1c*:** Whole blood samples were assessed for Glycated HB level with a micro-chromatographic procedure for the quantitation of glycosylated hemoglobin Glyco Hb Quick Column procedure (Helana).

Detection in Serum of IL-12 Levels: Serum samples were collected and stored at -4°C for the quantitative assessment of IL-12 using enzyme-linked immunosorbent assay (ELISA) technique (AviBion Human IL-12 EASIA kit). Samples were brought to room temperature and processed according to manufacturer's description. Colour detection was read at 450 nm Correcting for optical imperfections was done at 630 nm. A standard curve was plotted and results were calculated.

Statistical analysis
All data were analyzed using the Statistical Package for Social Science (SPSS) 14 for Windows. All data were presented as mean ± standard deviation (SD). Chi-square test (χ^2) was used to compare differences between the frequencies. Serum cytokines levels were analyzed using the normality test. Student t test was used to compare mean values between groups. Comparing several means was done by one-way analysis of variance (ANOVA) .Spearman rank correlation test was used for the assessment of correlation. The statistical significance was accepted as p value < 0.05.

RESULTS

The demographic and clinical data of the studied groups are enlisted in table 1. Table 2 shows levels of HbA1c, IL12, random blood sugar (RBS), and hematological parameters. Statistical analysis of the studied parameters in relation to Body Mass Index (BMI) are illustrated in tables 3. According to HbA1c levels, 12 patients only had good diabetic control, while 24 had poor control. Significant differences in IL12 levels are noted in relation to glycosylated hemoglobin levels (Table 4). IL12 levels were significantly higher in the recently diagnosed patients as demonstrated in table 5. Figure 1 shows the correlation between IL-12 and HbA1c.

Table 1. Demographic and clinical data of the studied groups.

	Cases (n = 50)	**Controls (n = 30)**	**Test of significance**	***P***
Sex (n, %) Male Female	 27(54.0%) 23(46.0%)	 15 (50.0%) 15 (50.0%)	χ^2=0.12	0.72
Age (yr)	9.19±3.3 4-14	8.7±3.8 3-14	*t* = 0.63	0.52
BMI	21.7±3.0 (15.1-29.5)	20.1±3.1 (15.2-26.4)	*t = 2.26*	0.026 *
Positive family history (n, %)	27/50(54%)	7/30(23.3%)	χ^2=7.22	0.007 *

*Significant BMI: body mass index

Table 2. Laboratoy data of the studied groups.

	Diabetic children	**Control**	***t***	***P***
WBcs $\times 10^3$/cm	8.3 ± 3.7	5.5 ± 1.5	2.18	< 0.01*
HB g/dl	12.2 ± 0.9	12.15 ± 0.97	0.31	0.6
HbA_{1c}(%)	9.47 ± 1.9 6.14 – 12.7	5.6 ± 0.36 5.1 – 6.2	10.8	< 0.001*
IL12 (pg/ml)	23.4 ± 10.79 10 – 56	6.2 ± 2.5 2 – 11	8.4	< 0.001*
RBS mg/dl	268.7 ± 95 116 – 466	87.9 ± 12.1 70 – 112	10.3	< 0.001*

*Significant ; IL-12: interleukin 12; RBS: random blood sugar; WBcs: white blood cells; Hb: hemoglobin; HbA1c: glycated hemoglobin.

Table 3. Statistical analysis of laboratory parameters in patients according to Body Mass Index (BMI)

	Underweight diabetics (n = 6) BMI<5th percentile	**Lean diabetics** (n = 36) BMI 5th-94th percentile	**Obese diabetics** (n = 8) BMI ≥ 95th percentile	***F***	***p***
IL-12 (pg/ml)	23.6±7.9	21.0±5.8	33.8±8.3	5.2	0.008*
HbA_1c (%)	8.7 ± 2.0	9.2 ± 1.87	11.0±1.4	3.63	0.03*
RBS mg/dl	233.3 ± 72.9	255.6 ± 81.1	354.2±86.3	4.54	0.015*
WBCs $\times 10^3$/cm	8.35 ± 2.8	8.6 ± 2.5	8.2±2.3	0.2	0.81

*Significant ; IL-12: interleukin 12; RBS: random blood sugar; WBcs: white blood cells; HbA1c: glycated hemoglobin.

Table 4. Statistical analysis of the studied parameters in relation to HbA1c%

	Good control (n = 12) HbA1c 6-7.9%	**Fair control** (n = 14) HbA1c 8-9.9%	**Poor control** (n = 24) HbA1c ≥10%	***F***	***P***
IL-12 pg/ml	16.8±6.6	22.4±7.1	27.3±7.7	4.1	0.02*
WBCs 10^3/cm	8.0±1.02	8.4±2.5	8.7±2.3	0.22	0.8
RBS mg/dl	141.3±19	247.9±25	344.5±64.1	79.9	<0.001*
BMI kg/m^2	20.76±2.3	21.8±2.98	22.1±3.3	0.79	0.45

*Significant ; IL-12: interleukin 12; RBS: random blood sugar; WBcs: white blood cells; BMI: body mass index.

Table 5. Statistical analysis of the relationship between the duration of diabetes and measured parameters

	< 1 year (n = 23)	**1-3 years (n = 18)**	**> 3 years (n = 9)**	***F***	***P***
IL-12 (pg/ml)	31.46±9	17.6±4.9	14.4±3.6	21.8	< 0.001*
WBcs $\times 10^3$	9.2±2.1	8±1.2	6.4±1.9	3.95	< 0.025*
RBS mg/dL	298.3±87	256.1±80.1	218.2±57	2.69	0.07
HbA_{1c} %	10.1±1.7	9.1±1.9	8.4±1.89	3.36	0.051

*Significant ; IL-12: interleukin 12; RBS: random blood sugar; WBcs: white blood cells; HbA1c: glycated hemoglobin.

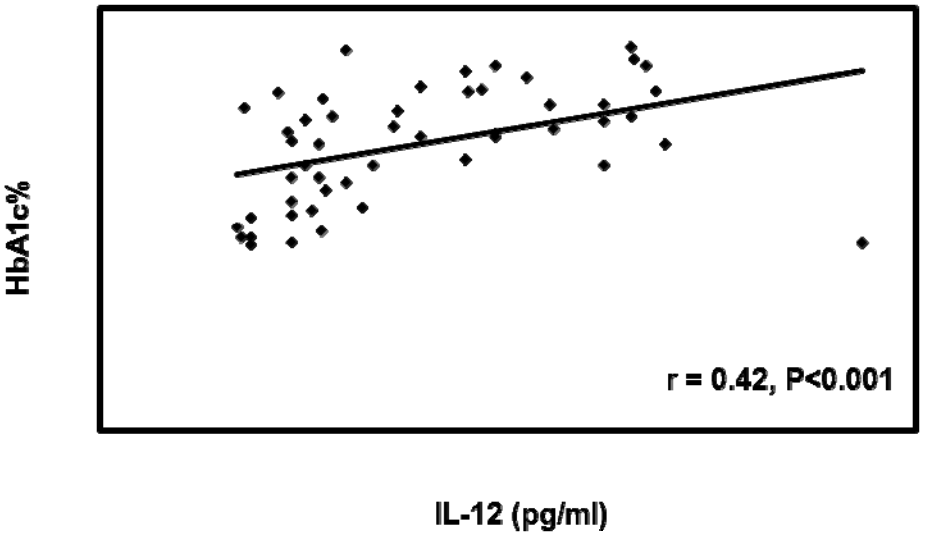

Figure 1. Correlation between IL12 levels and glycated hemoglobin (HbA1C).

DISCUSSION

The present study showed a highly significant increase in IL-12 level in diabetics (23.4±10.79 pg/ml.) as compared to healthy children (6.2±2.5 pg/ml). The same was reported by other researchers [7,8] . Blazhev et al.[9] proved that elevated IL-12 plasma levels were detected in both types of diabetes (T_1DM: X±SE, pg/ml = 2.4±0.16, T_2DM: 2.35±0.10, $p < 0.01$) as compared to healthy control subjects (1.86±0.07). Sarsvik et al.[10] suggested that IL-12 plays a critical role in the pathogenesis of type 1 diabetes as it affects a variety of stages in the immune response: it prompts NK cells and T cells to produce pro-inflammatory cytokines, as Interferon-γ (IFN-γ), IL-2, IL-3 and TNF-α; it contributes to NK cell maturation, and it stimulates CD4-CD25 T cell activation. IL-12 also regulates naive T cell differentiation into T-helper type 1 lymphocytes (Th_1), and inhibits differentiation into T-helper type 2 lymphocytes (Th_2) [11].

However, the precise mechanisms by which IL-12 could induce pathological damage in diabetics have not been completely clarified. The elevated levels of circulating IL-12 are associated with the activity or severity of various autoimmune diseases [12]. In patients with active multiple sclerosis (MS), serum levels of IL-12 are detectable in 53%, whereas none of the patients with clinically inactive MS has detectable IL-12. Moreover, IL-12 levels correlate well with the degree of inflammation in cerebrospinal fluid. In patients with rheumatoid arthritis (RA), levels of IL-12 are elevated in serum and synovial fluids with a direct correlation with disease activity[12].

Our results showed high production of IL-12p40 in patients with T_1DM. Gattorno et al.[13] proved the elevation of IL-12 in juvenile chronic arthritis, psoriasis, as well as in experimental models of diabetes. In agreement with an earlier study[14], our data revealed that there was no significant difference in IL-12 level in relation to gender in diabetic cases. Also we did not find significant difference between diabetic males and females with respect to HbA1c, CBC, BMI and RBS.

The study revealed a significant variation in IL-12 levels according to BMI in diabetics , as IL-12 levels were higher in overweight subjects (33.8±8.3 pg/ml) when compared with under and normal weight ones (23.6±7.9 pg/ml and 21 ± 5.8 pg/ml respectively). Increased levels of cytokines were reported in overweight patients with type 1 diabetes.[15] These findings can be explained by a proinflammatory effect of childhood obesity [8,9].

Similarly, the study showed a significant increase in HbA1c in over weight (11.0±1.4) versus lower level in lean (9.2±1.87) and underweight (8.7±2.0) diabetics. A previous study concluded that obesity and insulin resistance take part in the pathogenesis of type 1 diabetes in children and adolescents and said that over weight patients with type 1 diabetes had significantly higher HbA1c[15].

In agreement with Wu et al.[16], lower levels of IL-12 were noted in patients with good metabolic control (HbA1c%,6-7.9%) than in patients with fair control (HbA1c%,8-9.9) and poor control (HbA1c% ≥ 10) with $p<0.05$. IL-12 and other proinflammatory cytokines were reported to increase by hyperglycemia in subjects with

impaired glucose tolerance [17]. The highly significant correlation between IL-12 levels and HbA1c [r=0.42; p<0.001] which was consistent with a previous study[18] implies that the proinflamatory effect of IL12 may have a crucial role in the severity and control of T1DM.

In the present study and in a previous work [19], newly diagnosed patients with T_1DM of less than one year duration had significantly higher levels of IL-12 when compared to diabetic patient with duration more than one year. However; these results were inconsistent with those of Miteva et al.[20].

In conclusion, type 1 diabetes is associated with elevation of IL-12 levels. This association is more evident in both newly diagnosed and poorly controlled patients indicating a relevant role of IL-12 on the pathogenesis of the disease.

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